ON SOME PROPERTIES OF THE JELLY COAT IN OOCYTES AND MATURE EGGS OF SEA URCHINS. A STUDY OF PHASE-DEPENDENT CHANGES OF METAPLASMIC LAYERS IN THE CELL SURFACE

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The maturation of the oocyte of the sea urchin involves a number of complicated processes. The displacement of the cortical granules is one of them (Monné and Harde, 1951). The whole cytoplasm of the oocyte incorporates $^{35}SO_4$; during the course of maturation the labelled material is displaced toward the cortex (Immers, 1961a). For many years this writer has observed that there are considerable differences between the jelly coats of oocytes and maturing eggs in comparison with those of mature eggs. In the writer's studies, these differences revealed themselves in the way of interaction between sperm and the jelly coat of the egg. A brief survey will be given of some of the work that has been done concerning these interactions.

Hartmann and Schartau (1939) (see also Hartmann, 1956) demonstrated that a concentrated sperm suspension is able to dissolve the jelly coat of *Arbacia lixula*. They showed that the sperm liquor has a similar effect.

Vasseur (1951) also used living sperm but extracted the substance of the jelly coat of the egg by means of acid sea water (pH 5.2). The changes in viscosity of the jelly solution were measured after addition of sperm. They cause an increase in viscosity of the jelly solution. If, however, some octylalcohol is added, either before or different times after insemination, there is a sudden considerable drop in viscosity of the jelly that is not observed upon addition of octylalcohol alone. According to Vasseur the octylalcohol removes certain substances that are released when bringing the jelly coat in solution. These substances should inhibit an enzyme contained in the sperm. According to Vasseur these inhibitory substances are masked in the intact jelly; therefore a splitting of the intact jelly coat occurs upon penetration of spermatozoa. In some other investigations the splitting action of sperm extracts was tested on jelly solutions or on hyaluronate. Monroy and Ruffo (1947) and Lundblad and Monroy (1950) found a weak action on hyaluronidate but this mucopolysaccharide is not present in the jelly. A viscosity-lowering effect on jelly coat solution by sperm extract on its own species was described. The sperm extract was shown to have a proteolytic activity which may split the protein moiety of the jelly. Sensitivity of the active principle to boiling pointed to an enzyme effect. A precipitation of the jelly by basic substances present in the sperm extract may also have played a role in the investigation of Lundblad and Monroy (1950). Hultin and Lundblad (1952) demonstrated the presence of some carbohydrases in sperm extract. Hultin *et al.* (1952) introduced a method of testing the removal of the jelly coat from the eggs of *Arbacia lixula* (Naples). The spermatozoa were spun down at 6000–7000 *g* for 5 minutes and the supernatant (S1) was removed. The spermatozoa were resuspended in sea water and the centrifugation repeated. The supernatant (S2) was removed. Usually five supernatants were prepared in this way (S1-S5). The supernatants were added to an egg suspension in sea water and the sedimentation of the eggs was observed in concave dishes (Boveri dishes). The sedimenting eggs were collected in the center of the dish and it was easy to decide macroscopically as to whether the eggs had lost their jelly coats or not. These were lost by the action of S1 and S2, but later the dissolution was replaced by a precipitation of the jelly coats. This was caused by the extraction of a "jelly-precipitating factor" from the sperm (sperm antifertilizin). Hultin *et al.* proposed the abbreviation "Je Ppt F (Sp)" for this factor, "Je Ppt F(E)" being the corresponding factor from the egg. The jelly-dissolving factor from sperm was inactivated by heating to 60° for 30 minutes. This speaks in favor of its enzyme character. After heat treatment of the sperm extracts the jelly-precipitating factor became more active. This shows that the jelly-dissolving and the jelly-precipitating factor exist beside each other in perhaps all the supernatants S1-S5, but, depending on concentrations, they weaken or exclude each other. Hartmann and coworkers (see Hartmann, 1956) considered that the jelly-dissolving and -precipitating effect was due to the sperm factor "Gynogamon 2." The results reported indicate that this gamone must be separated into two factors.

Owing to outside circumstances the investigations of Hultin *et al.* concerning the jelly-dissolving factor could not be further elaborated but the results obtained by Hathaway *et al.* (1960) and Hathaway and Warren (1961) are rather analogous. Substances may be released from the surface of sperm of *Arbacia punctulata* (Woods Hole) by treatment with homologous jelly substance "fertilizin" or with 10^{-4} *M* sodium lauryl sulfate. These extracts caused a complete disappearance of the jelly coats surrounding the *Arbacia* eggs. The activity is destroyed by heating for 5 minutes to 80°. This corresponds well with the degree of thermolability found for the jelly-dissolving factor studied by Hultin *et al.* In the experiments of Hathaway *et al.*, the presence of jelly-precipitating factor had the same disturbing effect on the demonstration of the jelly-dissolving factor as was reported by Hultin *et al.* Hathaway *et al.* reported that they could spin down the jelly-precipitating activity. This corresponds to the results obtained by Runnström *et al.* (1955). They found the jelly-precipitating factor in the microsome fraction but also in the supernatant. A treatment of the microsome fraction with ribonuclease caused a further release of jelly-precipitating factor.

In summary it may be said that there is evidence of an enzymatic activity in the sperm that dissolves the jelly coat of the egg. The knowledge both of the character of this enzyme and the extent of the splitting is unsatisfactory. The splitting involves probably a certain not too deep-going depolymerization. The splitting enzyme may be present both in the spermatozoon and in the sperm liquid. Aketa (1961) demonstrated that the jelly coat does not play a role in the acid formation ensuing upon fertilization of the sea urchin egg. This is in keeping with the view that the breakdown of the jelly coat occurring under the impact of the spermatozoa does not lead to a pronounced splitting (scission) of the jelly coat substance. The question should, however, be reinvestigated with rather heavy sperm suspensions.

The "Je Ppt F(Sp)" is obviously a basic protein (T. Hultin, 1949); the same seems to hold for J Ppt F(E), as the results of Runnström and Monroy (1950) indicated. Hultin *et al.* (1952) regarded J Ppt F(E) as identical with the membrane-toughening factor of Motomura (1950), who gave the final demonstration of the basic character of this substance which he designated as "colleterin" (1957). This writer would prefer to maintain the descriptive designation, fertilization membrane-toughening factor, abbreviated, *e.g.*, Fmb tough F(E).

A change in the physical character of the jelly coat was also brought about by addition of certain substances, like heparin (Runnström and Wicklund, 1950; Harding, 1951) and the potent fertilization inhibitor from *Fucus*, Fe Inh (Fu) (see Harding, 1951; Runnström and Hagström, 1955; Esping, 1957a, 1957b; Branham and Metz, 1959; and Metz, 1961). The Fe Inh (Fu) counteracts the breakdown of the substance of the jelly coat by spermatozoa. Removal of the jelly coat reduces the inhibitory action of Fe Inh (Fu). The treatment of the naked egg with the inhibitor still brings about a considerable inhibitory effect (Runnström and Hagström, 1955). The same was the case with heparin (Harding, 1951). In view of the advantage of working with defined agents, chondroitin sulfate and germanin were also tested and were found to act as fertilization inhibitors in a manner very similar to that of Fe Inh (Fu) (Wicklund, 1954; Hagström *et al.*, 1957). Chondroitin sulfate impairs, for example, the dissolution of the jelly coat by spermatozoa.

This brief survey shows that the jelly coat may be affected by two different groups of substances, namely (1) by basic proteins, (2) by substances of acid character that are able to cross-link the fine structure of the jelly coat. These substances inhibit the dissolution of the jelly coat and delay the penetration of the spermatozoa. In the present paper it will be demonstrated that the jelly coat has different properties in different phases of development of the female genital cell. Furthermore an attempt will be made to explore the mechanism of these changes, both by evidence reported below and by evidence from pertinent literature. It will follow that lively interactions occur between so-called extraneous coats and the cytoplasm.

Material, Methods

The egg and sperm of *Paracentrotus* (Pa.) *lividus*, *Arbacia* (A.) *lixula* and *Psammechinus* (Ps.) *microtuberculatus* and *Ps. miliaris* served as the main material in this research. The three first mentioned species were made available at the Stazione Zoologica, Naples, the last mentioned at the Kristineberg Zoological Station (Swedish west coast). The ovaries were cut open and the outflowing eggs were collected in sea water and thereafter passed through bolting silk. The eggs were further washed by suspending them in 300–500-ml. beakers. The sperm was removed from the testicles without dilution and then brought into small tubes that were stoppered and kept in a refrigerator. Two drops of the dry sperm were suspended in 5 ml. sea water and further diluted according to the type of experiment carried out (1 : 5; 1 : 10 etc.). An egg suspension was inseminated by rapid mixing with the diluted sperm. The jelly coat was removed from the eggs either by treatment with acid sea water (pH 5.2), following the direction of Vasseur (1948), or by filtration through gauze under pressure as described by

Markman (1958). This latter method was also used in order to estimate the mechanical resistance (consistency) of the jelly. In the experiments with basic stains or protamin sulfate the mixture of egg suspension and solution of the substances was made in a shallow concave dish but the observations were made after putting the oocytes or eggs between slide and coverslip. Between the observations, the slides were kept on stands enclosed in a container in which the atmosphere was kept moist. The stains used were brilliant cresyl blue (E. Merck) and toluidine blue (Gurr), the protamine sulfate was from Light and the reduced glutathione from Eastman & Co. A Leitz polarization microscope, furnished with a Köhler compensator, was used for the observations of the positive or negative sign of the birefringence with reference to the radial direction of the egg. The size of the figures (phase contrast) is *ca.* 340 × object.

The oocytes of the first order will be designated below as "oocytes" or "resting oocytes," whereas oocytes of the second order will be called "oocytes in maturation divisions" or "maturing oocytes."

For convenience the designation, "mature eggs," will be used for those which have completed the maturation division (meiosis); on the other hand the designations, "underripe," "ripe," etc. will refer to the changing state of cytoplasm that may be observed after meiosis.

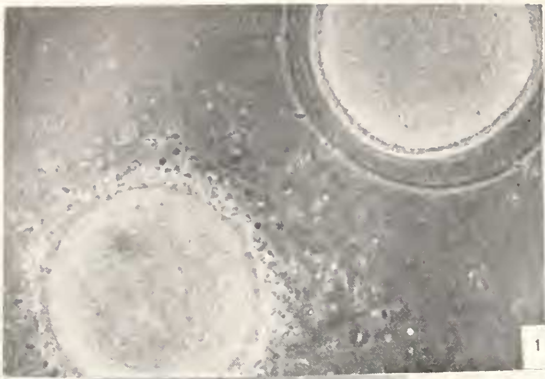
RESULTS

a. Effect of the sperm on the jelly coat of the egg

In one experiment, eggs of *Ps. microtuberculàtus* were washed repeatedly and then inseminated with a heavy sperm suspension, $5\text{–}6 \times 10^6$ per ml. The spermatozoa attacked the jelly coats of the mature eggs as in the experiments of Hartmann and Schartau (1939) on *Arbacia* eggs. In the present experiment, 70–80% of the eggs had lost their jelly coats 10–15 minutes after insemination. In the eggs retaining their jelly coats, very few spermatozoa were present. These were able to move around and in some instances actually left the jelly coat. After the treatment, the jelly coat offered a low resistance to the movements of the spermatozoa. The same phenomenon was readily observed also in the other species studied in this respect, *Ps. miliaris* and *Pa. lividus.*

In contrast, the oocytes did not loose their jelly coats on insemination. Great numbers of spermatozoa were caught in the oocyte jelly coats, particularly in the inner zone. There were fewer spermatozoa in an outer zone (see Figure 1, representing an oocyte of *Pa. lividus*, about 30 minutes after insemination). The width of the jelly coat was about 50 μ (diameter of the oocyte is 94 μ). In the same figure a mature fertilized egg is represented. Very few spermatozoa were present in the jelly coat of the egg. Figure 2 gives another rather typical picture of an oocyte with accumulation of spermatozoa.

The spermatozoa invaded the jelly coat of the oocyte without difficulty. A number of them penetrated to the surface of the oocyte and there caused the formation of reception cones, the structure and behavior of which were recently examined by Runnström (1963); see that paper for references to previous literature. Upon heavy insemination, most of the spermatozoa were immobilized in the jelly coat. The heads of these spermatozoa often became spherical; the middle piece and tail remained unchanged so far as can be seen with the light microscope.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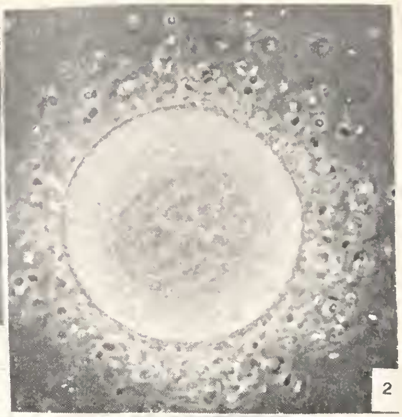
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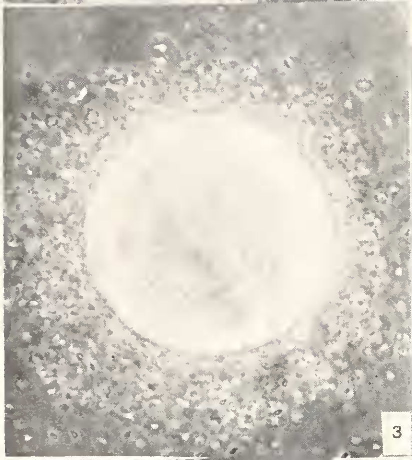
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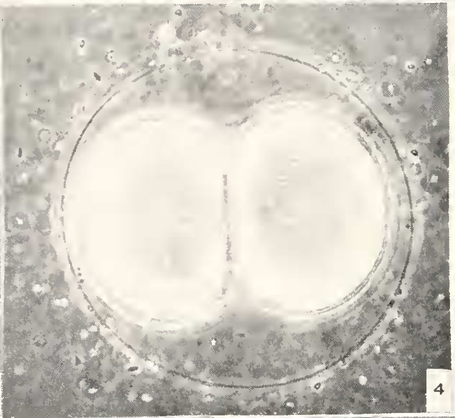
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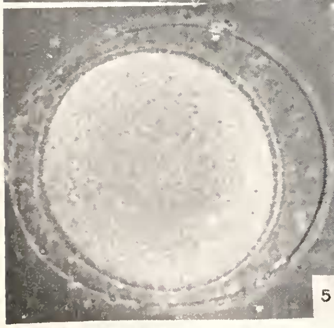
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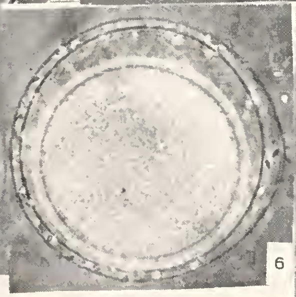
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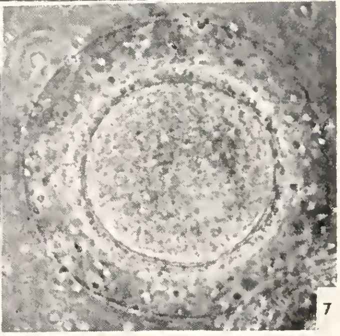
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FIGURE 1. An oocyte and a ripe egg of *Paracentrotus lividus;* 30 minutes after insemination. 310 ×.

FIGURE 2. Oocyte of *Paracentrotus lividus, ca.* 25 minutes after insemination. 330 ×.

FIGURE 3. Oocyte of *Psammechinus microtuberculatus* in meiotic division. Penetration of several spermatozoa has provoked the formation of mitotic figures. 325 ×.

FIGURE 4. Egg of *Psammechinus microtuberculatus* about 70 minutes after insemination. 325 ×.

FIGURE 5. Egg of *Paracentrotus lividus,* four minutes after fertilization. 350 ×.

FIGURE 6. Eggs of *Paracentrotus lividus,* two minutes after insemination, exposed to 0.01% brilliant cresyl blue, reception cone visible in the proximal region of the egg. 310 ×.

FIGURE 7. Oocyte of *Paracentrotus lividus,* two minutes after insemination, exposed to 0.01% brilliant cresyl blue; photographed 35 minutes later. 310 ×.

Within the jelly of the oocyte, the spherical heads showed a dark outline in phase contrast. Such spermatozoa were present in the oocytes represented in Figures 1 and 2.

Since Selenka (1878), it has been known that the jelly coat of sea urchin oocytes is pierced by very thin filaments, the function of which seems to be the connection of the oocytes with the follicle cells; possibly they mediate uptake of substances from the follicle cells (see further Lindahl, 1941, and Runnström, 1944). The filaments are evidently withdrawn or broken down before the maturation divisions. The presence of the filaments does not exclude penetration of spermatozoa to the egg surface with subsequent formation of reception cones and immobilization of the spermatozoa. Despite the presence of filaments the situation was thus similar to that represented in Figure 1. The thin reception cones (*cf.* Runnström, 1963, Fig. 5) may also penetrate the whole width of the jelly coat. In view of these facts, the jelly coat of the oocytes seems to be rather highly organized. It may consist of a rather rigid jelly pierced by channels through which filaments, narrow reception cones and spermatozoa may pass. The rather high rigidity of the jelly coat of the oocytes was demonstrated by the method of Markman (1958). Non-fertilized eggs of *Paracentrotus* were filtered under light pressure through gauze, the meshes of which were smaller than the diameter of egg + jelly. The presence or absence of the jelly coats was tested by bringing a concentrate of non-fertilized eggs on a slide. The surfaces of the eggs without jelly coats were in close contact, whereas those with jelly coats were separated by an interspace representing the width of the jelly coats. In several such experiments 90–99% of the mature eggs had lost, whereas all the oocytes present had retained, their jelly coats. It was ascertained that the jelly coats of the mature eggs showed no strong swelling; the width of jelly coat was approximately equal to the radius of the oocyte or mature egg.

In one experiment (January, 1963) 237 non-inseminated female gametes of *Ps. microtuberculatus* were examined among a still greater number that had passed the gauze filter; out of the sample examined, 46 (19.5%) were oocytes with intact jelly coats, 189 were mature eggs, of which 156 (82%) had lost the jelly coat. The percentage of mature eggs, the jelly coats of which resisted filtration, shows that these eggs belonged to a batch of a moderate degree of cytoplasmic underripeness.

The greater resistance of the jelly coats of the oocytes was observed also when the jelly coats of egg suspensions were repeatedly washed with sea water under much agitation.

Oocytes in maturation divisions have not yet developed the complete mechanism that protects against polyspermy (Brachet, 1921; Runnström and Monné, 1945). In some experiments, counts of the number of reception cones were tried; it could be roughly estimated that the number of reception cones formed in maturing oocytes corresponded to about one-tenth of those found in resting oocytes. The relation between spermatozoa and jelly coat in maturing oocytes is similar to that described for the resting oocytes. There is a strong accumulation of immobilized spermatozoa in the jelly coat. Sometimes they are more numerous in an inner zone of the jelly coat, as in the case of resting oocytes; more often, however, they are rather uniformly distributed in the jelly coat of the maturing oocyte, as shown in Figure 3 (67 minutes after insemination). In this instance also, the sperm heads often became spherical. Figure 4 represents a mature egg about 70 minutes after fertilization. It belonged to the same egg batch as that of the maturing egg of Figure 3. The jelly coat of the egg of Figure 4 contained fewer spermatozoa than the resting or maturing oocytes; moreover, they retained a certain mobility and none of them had assumed a spherical form. Nevertheless, eggs of this category were considered not to have attained full cytoplasmic ripeness. As described earlier (see Runnström, 1949; B. Hagström, 1955) for *Ps. miliaris* from the Swedish west coast, a state of underripeness of the eggs was often observed during the breeding season, June–August. The characteristic of this state is a low rate of fertilization (B. Hagström, 1955) partly due to an accumulation and immobilization of the spermatozoa in the jelly coat. In extreme cases the fertilization was blocked in a varying number of eggs.

A relatively mild form of underripeness may be present for a great part of the breeding season at Kristineberg. In these cases the eggs have a low rate of fertilization the first hour after removal of the eggs from the ovaries but gradually the rate of fertilization increased and attained a maximum 3–4 hours after removal from the ovaries (B. Hagström, 1955).

The littoral so-called Z-form of *Ps. miliaris* (Lindahl and Runnström, 1929) is exposed to variations in salinity and temperature. These external factors probably influence the rate of cytoplasmic ripening in this form. The so-called S-form of *Ps. miliaris* (from a depth of 30–40 meters) is living under more constant external conditions. The state of underripeness is limited here to the beginning of the breeding season. It may be found that in the latter half of August the ovaries contain plenty of eggs having undergone nuclear maturation that are not fertilizeable by highly motile spermatozoa from S- or Z-form. In *Ech. cordatum* analogous conditions were found; (the latter half of July is the period of cytoplasmic underripeness with full ripeness following in the early half of August). These indications are based on experience from several years but there may of course be variations with respect to dates and duration of the periods of cytoplasmic maturity.

Phenomena comparable to those observed in Kristineberg were revealed in observations on *Pa. lividus* and *Ps. microtuberculatus* carried out at the Stazione Zoologica, Naples, in December, 1960 and 1962. During this period the filtering of the eggs under pressure deprived the eggs of their jelly coats only to a limited extent. Also, during the spring season it was observed that accumulation of spermatozoa in the jelly coat occurred; concurrently the resistance against filtration was high. It seems natural to consider the state of the jelly coat during the period of underripeness as directly continuous with the state found in the oocyte and in the stage of meiotic divisions. The duration of the period of cytoplasmic underripeness is dependent on exterior conditions. Lindahl and Runnström (1929) found reasons to believe that in *Ps. miliaris* the sexual maturity was dependent on the average temperature during a certain period rather than on peaks of temperature.

A moderate accumulation of spermatozoa may occur within the jelly coat but the fertilization membrane may nevertheless be elevated from the egg surface.

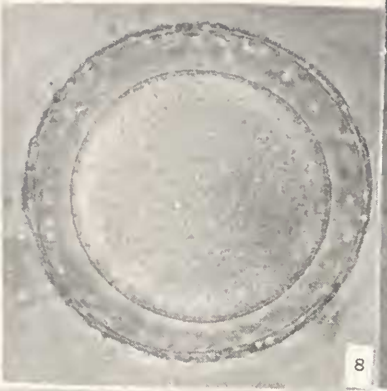
Such a case is represented in Figure 5 (*Pa. lividus*). Four minutes after insemination the fertilization membrane was, however, rather asymmetric; at the "proximal" pole (Runnström, 1962), the fertilization membrane was more elevated and more refractile than at the "distal" pole. Moreover, it was observed how the spermatozoa gradually left the jelly coat. There is an evident change in the property of the jelly coat starting from the moment when the proximal part of the fertilization membrane becomes smooth. As previously demonstrated (Runnström, 1962), this smoothing is dependent on the formation of the proximal concavity within which the penetrating spermatozoon is usually attached. The concavity formation is probably the expression of the secretion of a factor(s) that brings about the final incorporation of the cortical lamella (see Endo, 1961), and the final detachment of the fertilization membrane from other cortical elements and from the egg surface. At the distal pole, on the other hand, the fertilization membrane was less refractile and seemed granular, which signifies that the contact with the egg surface was not definitely broken (electron microscopic observations). In many cases the state represented in Figure 5 was only temporary and the smooth state of the fertilization membrane spread also to the distal side and simultaneously spermatozoa were released from the grip of the stiff jelly coat. These frequently repeated observations indicate that a secretion spreads from the proximal side of the egg in distal direction. This brings about the final detachment of the fertilization membrane from underlying components. The same secretion also changes the jelly coat so that its consistency decreases. The rate of smoothing of the distal region of the fertilization membrane varies in different batches of eggs. This may also be due to a certain state of the cytoplasm which depends in the last instance on exterior conditions. These may prolong or shorten a state of underripness. The fertilization membrane-toughening factor of Motomura (1957) is probably superimposed on the smoothing factor. The progress of jelly-toughening was followed with different methods by Motomura (1950) and by Markman (1958).

b. Precipitation of the jelly coat with basic stains

Oocytes and eggs of *Pa. lividus* were inseminated with a heavy sperm suspension. Two minutes later brilliant cresyl blue was added so as to make the suspension about 0.01% with respect to dye. There was an immediate precipitation of the jelly coat with a strong contraction (see Fig. 6). The spermatozoa that were present in the jelly coat were occluded and immobilized.

In some experiments the jelly coat shrunk down to a still thinner layer outside the fertilization membrane. In oocytes, a precipitation of the jelly coat was also manifested by a marked outline; on the other hand, there was no, or a very slight, contraction of the jelly coat, which remained many times thicker than the jelly coat in the fertilized egg. This is evident from a comparison of Figures 6 and 7. The latter represents an oocyte 37 minutes after insemination when the sperm-induced protrusions (see Runnström, 1963) have been retracted.

When toluidine blue dissolved in sea water was the jelly-precipitating agent the results were similar to those described. The concentration used was generally 0.01%. When the mature eggs were kept between slide and coverslip, they be-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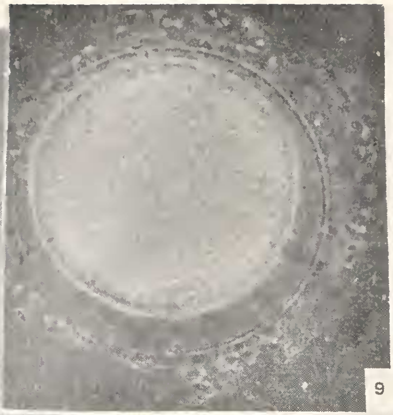
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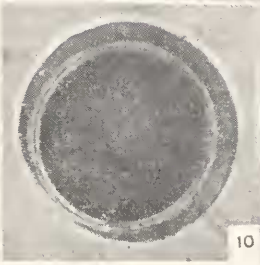
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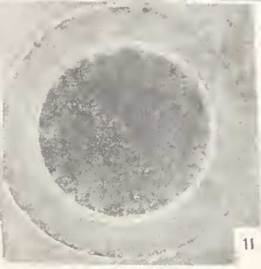
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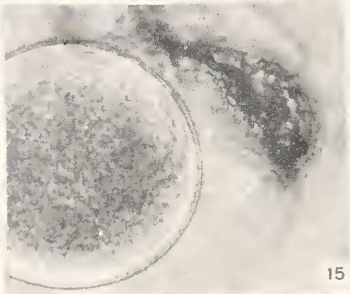
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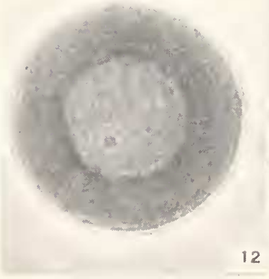
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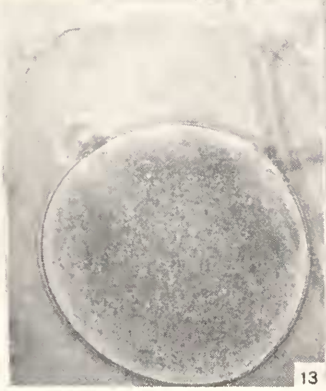
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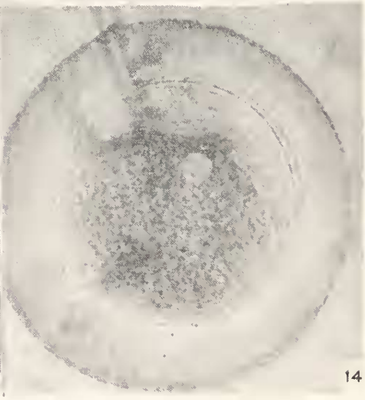
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FIGURE 8. Egg two minutes after insemination, exposed to 0.01% toluidine blue in sea water, kept aerobically for 70 minutes. 380 ×.

FIGURE 9. Egg of *Paracentrotus lividus* two minutes after insemination, exposed to 0.01% toluidine blue, kept for 70 minutes under increasingly anaerobic conditions. 380 ×.

FIGURE 10. An unfertilized egg of *Arbacia lixula* brought into a 0.01% solution of brilliant cresyl blue in sea water; photographed *ca.* 25 minutes later. 310 ×.

FIGURE 11. A fertilized egg of *Arbacia*, two minutes after insemination brought into a 0.01% solution of brilliant cresyl blue in sea water; the egg belonged to the same batch as the unfertilized egg in Figure 10. 310 ×.

FIGURES 12, 13 and 14. Non-inseminated resting oocyte, oocyte in meiosis and mature egg of *Psammechinus miliaris*, immersed in 0.004% toluidine blue in sea water and kept for 15 minutes under aerobic conditions. 350 ×.

FIGURE 15. Unfertilized egg of *Psammechinus miliaris* with contracted fibrous jelly coat subsequent to addition of a 0.02% solution of brilliant cresyl blue from the side of the coverslip. 350 ×.

haved in two different ways (1) the precipitated state of the jelly coat continued; or (2) a swelling of the jelly coat occurred. It was easily stated that the first corresponded to a maintained oxidized state of the dye (see Fig. 8). This was found when the eggs were located near the edge of the drop of egg suspension enclosed between slide and coverslip. In eggs with a more central location in the drop, the dye was reduced and swelling of the jelly coat occurred as a consequence (see Fig. 9). Eggs with precipitated jelly coats were often aggregated; after reduction of the dye the eggs separated.

The precipitated jelly showed a birefringence that was positive in the radial direction. Monné (1943) previously demonstrated that after addition of acridine orange, the jelly coat has a similar birefringence. The sign of the birefringence that develops after staining is opposite to that of the fertilization membrane. In the egg represented in Figure 8 neither the membrane nor the jelly coat showed any birefringence. By the strong contraction of the jelly, this was amalgamated with the fertilization membrane to such an extent that the two opposite birefringences canceled each other out, the retardations being approximately equal. If, on the other hand, the contraction of the jelly was weaker, one could distinguish the positive birefringence of the jelly coat as contrasted to the negative birefringence of the fertilization membrane. The thin border of the jelly coat of the oocyte was birefringent; the character of the birefringence was the same as in the mature egg. When the jelly coat swelled under anaerobic conditions (see Fig. 9), the birefringences disappeared. The coverslips were removed in one experiment of the kind just described. Due to the contact with air, the dye was reoxidized and a reprecipitation of the jelly coat occurred. Under such circumstances the precipitate often took the form of threads appearing in the medium surrounding the egg.

After the addition of the dye, the precipitation of the jelly coat of mature unfertilized eggs was less pronounced than in the previously fertilized eggs. While in these latter the jelly coats shrank to a narrow rim in 2–3 minutes after addition of the stain, the shrinkage was slower and more gradual in the mature non-fertilized egg; they could, for example, decrease in width by 20–30% in 10–15 minutes. The difference could be due to the presence of spermatozoa within the jelly coat of the fertilized eggs. These may, for example, give off their jelly-precipitating factor. On the other hand, the shrinkage is to a large extent reversible after the reduction of the dye. Moreover, the spermatozoa occluded in the jelly coat of fertilized eggs are not damaged as far as can be judged from their appearance. Nevertheless it cannot be excluded that the jelly-precipitating factor from sperm plays a certain role, particularly after the rather heavy insemination in the experiment to which the eggs in Figures 8 and 9 belong.

The different behavior of fertilized and unfertilized eggs could be studied with advantage in eggs of *Arbacia lixula*, in which the fertilization membrane is only slightly elevated upon fertilization. Eggs from the same female of *Arbacia lixula* were divided into two portions. One (a) was directly transferred to 0.01% brilliant cresyl blue in sea water; the second (b) was inseminated with a very weak sperm suspension and transferred two minutes later to the stain solution. Figures 10 and 11 represent one unfertilized and one fertilized egg that were brought together on the same slide and photographed *ca.* 25 minutes after being transferred to the dye solution. Under aerobic conditions, the fertilized egg became as usual more heavily stained. It is of interest to note that this was true for the jelly coat as well. In the non-fertilized egg both the cytoplasm and the jelly coat were only faintly stained. The contraction of the jelly coat was weaker than in the fertilized egg. In many cases the contraction of the jelly of the fertilized egg was even stronger than shown in Figure 10, so that the jelly coat formed a thin rim outside the membrane. In this and in other experiments any effect of the jelly-precipitating factor from the sperm was insignificant.

In a similar experiment, the fertilized portion of eggs was transferred to the dye solution 23 minutes after insemination. The eggs, including the jelly coats, were measured with a screw micrometer, beginning 7 minutes after transfer to the stain solution. No essential changes in diameter took place during the measurements. The difference noted depended wholly on the different degree of retraction of the jelly coat. The difference between the fertilized and unfertilized eggs should in fact have been greater, because in the fertilized eggs the width of the perivitelline space is included in the measured diameter. In *Arbacia*, however, this space is so narrow that it can be disregarded.

The following averages (micrometer scale units, each equal to 0.25 μ) were found:

Unfertilized mature eggs	406.6 ± 9.3	($n = 18$)
Fertilized eggs	349.4 ± 9.3	($n = 16$)

The difference and its standard error is 57.2 ± 13.2. The probability that the two groups of eggs belong to the same category is thus <0.01.

The capacity of the jelly of the oocyte (of 1st and 2nd order) to take up stain seems to be lower than in the mature egg. Moreover the stain may become reduced sooner than in mature eggs. In contrast, the cytoplasm of the oocyte is more heavily stained than in subsequent stages. The capacity of the cytoplasm to take up dye is evidently very high, whereas the reduction is slow, owing to this high capacity of uptake. Evidently the jelly coat contains dehydrogenases or is in contact with reducing systems contained in the egg surface, the effect of which becomes weaker after maturation. Probably, the degree of staining of the jelly coat depends primarily on its capacity of binding the dye. This capacity increases after the maturation divisions and again after fertilization. In the fertilized eggs the occluded spermatozoa may play a role for the reduction of the dye.

Continuous observations were made on some female gametes of *Ps. miliaris* that were immersed in 0.004% toluidine blue in sea water.

After 10–14 minutes, no rim was visible in the jelly coat of the oocyte (Fig. 12). In a maturing oocyte (Fig. 13), a rim was present but the jelly showed very little shrinkage. Finally, in a mature egg the width of the jelly coat had undergone a shrinkage of about 30%; a marked rim had appeared (Fig. 14). The staining was strongest in the mature egg. In the latter there was a tendency to formation of fibers. This was particularly pronounced if the dye was added from the side of a suspension of eggs enclosed between slide and coverslip (see Fig. 15). The jelly coat contracted and its bulk accumulated on the side of higher dye concentration. A rather dense framework of fibers appeared. The single fibers were not uniform. In the fibers two components are distinguishable in phase contrast. The one is thinner and strictly fibrous and the other is granular. In more contracted fibers the granules (diameter *ca.* 0.2 μ) became more closely adjacent and broadened slightly in a transverse direction. The granules may possibly correspond to sites with higher concentration of acid groups, which thus become centers of contraction under the effect of the basic stain. When fertilized eggs were stained from one direction, the framework of fibers in the jelly coat was usually still denser than in the non-fertilized eggs. Conversely, oocytes treated in the manner described showed a framework in which the contraction is much less than in the mature eggs. The fibers are more granular and the meshes larger.

The stained unfertilized eggs were inseminated. Although many spermatozoa were unable to penetrate the jelly, the eggs were fertilized, but no membrane elevation took place. On immersion of the eggs in hypertonic medium (2 ml. sea water + 0.5 ml. 2.5 N NaCl), a radially striated hyaline layer, some microns wide, appeared, constituting a reliable indication that the eggs were fertilized. The fertilization membrane covered the hyaline layer, but no birefringence was demonstrated in the fertilization membrane. In the course of 20–30 minutes, however, a gradual delamination of the two membranes occurred. This was particularly obvious after immersion of the eggs in hypertonic medium. Simultaneously, the fertilization membrane acquired its birefringence. Under normal conditions this occurs as early as one minute after fertilization. The treatment of eggs with basic dyes thus causes a considerable delay of delamination.

In certain cases the previous staining interrupted the spreading of the activation impulse so that only the region encompassing the site of sperm entry was fertilized.

c. Effect of protamin on the jelly coat

It is well known (see Runnström, 1949, p. 294) that the jelly coat surrounding the sea urchin egg is precipitated under the influence of basic proteins, *e.g.*, protamins. Such experiments were repeated with protamin sulfate. Eggs of *Pa. lividus* were inseminated and, two minutes later, brought into a 0.005% solution of protamin sulfate in sea water (pH regulated to about 8.0). After 2–3 minutes the jelly of the mature fertilized eggs had contracted to a thin rim covering the fertilization membrane. Spermatozoa were captured in the contracting jelly.

The jelly coat of inseminated oocytes behaved rather differently upon addition of protamin. A precipitated outline was visible at the border of the jelly coat, but there was no, or only a slight, contraction of the jelly coats which, as usual, contained numerous spermatozoa. Whereas the contracted jelly coat of the fertilized eggs had a width of about 2 μ, the jelly coats of oocytes were about 20–25 μ wide. In some of the oocytes no precipitation of the exterior outline was found. The same striking difference appears here between oocytes and mature eggs as in the experiments with dyes. The jelly coat of the non-inseminated did not differ appreciatively from that of inseminated oocytes.

In further experiments a lower concentration of protamin sulfate was used, *viz.* 0.001–0.0005%. In these the contraction process was slower than at the higher concentration of protamin sulfate but the end result was similar. It was now evident that the jelly coat of unfertilized mature eggs did not contract to the same extent as that of fertilized eggs. On the other hand, the contraction was considerably stronger than in the non-inseminated oocytes. In one experiment (1) oocytes, (2) unfertilized mature and (3) fertilized eggs of *Pa. lividus* were exposed to 8×10^{-4}% protamin sulfate for 8–10 minutes. After this time the width of the jelly coat was, in (1) 46 μ, in (2) 20 μ; in (3) the jelly coat formed a narrow rim. Measurements were made on three eggs of (1) and (2), respectively. Qualitative observations on a great number of eggs confirmed the results of measurements. Even when the jelly coat of unfertilized eggs showed a visible precipitated outer rim, certain spermatozoa were usually able to penetrate to the egg surface and bring about fertilization of the mature egg, whereas the penetration of spermatozoa into the jelly coat of oocytes did not seem to be hampered.

Usually the fertilization membrane did not elevate from the egg surface with the exception of a region around the site of penetration of the spermatozoon. In this region delayed plate- or rod-like cortical lamellae were often present in the perivitelline space (Runnström, 1948; Endo, 1952). When glutathione was added in the concentration of 0.015% the elevation of the fertilization membrane was more pronounced, even in the presence of protamin sulfate. The addition of glutathione seems to increase the formation of plates and rods that are thinner and more elongated than in the parallel test containing protamin sulfate only. Exposure of unfertilized *Paracentrotus* eggs to protamin (0.005–0.012%) interferes rather strongly with the development of the eggs. The exposure lasted, *e.g.*, for 30 minutes; the eggs were then washed and fertilized in pure sea water. The blastocoel was narrower than in the control eggs and also the further development showed a considerable delay and inhibition, whereas the development was rather normal in eggs that were fertilized and, 5 minutes after fertilization, immersed in the protamin solution; the exposure here also lasted for 30 minutes. The eggs exposed to protamin before fertilization showed stronger damage in the vegetal, as compared with the animal, region in which a certain enlargement of the acron and the ciliary tuft was often observed. In some tests the eggs were exposed to a protamin solution that in addition contained glutathione (0.015%) during the exposure of unfertilized eggs. Subsequent to fertilization the development of these eggs became more normal than after pretreatment with protamin alone. In the experiments briefly mentioned it was irrelevant whether the eggs were surrounded by a jelly coat or if the jelly coat had been removed by a previous exposure to acid sea water.

DISCUSSION

The main observation reported in the present paper is the different behavior of the jelly coat toward added spermatozoa in oocytes or maturing oocytes on one hand, and mature eggs on the other. The spermatozoa are not able to depolymerize the jelly coats in the first-mentioned stages, whereas this occurs in the mature eggs. However, there is a stage of underripeness prevailing after the meiotic division. This stage may last for a longer or shorter period, according to the species and external conditions. During the time of underripeness the spermatozoa accumulate in the jelly coats, which are evidently very resistant to the depolymerizing action of the spermatozoa. This state is gradually changed into the fully ripe one. This transition may be observed in individual batches of eggs or in eggs from different females during the progress of the breeding season.

It is very probable that the process of cytoplasmic ripening runs continuously until a certain maximum has been attained. Perlmann (1956) found that the rate of fertilization in *Pa. lividus* from Naples increases during the spring to attain a maximum in June. Before this period the removal of the jelly coat brought about an increase in rate of fertilization, but during the optimum period, the rate did not depend on the presence or absence of the jelly coat, *i.e.*, the resistance offered by the jelly coats had a minimum. In all these experiments care must be taken that the spermatozoa are in optimum conditions (see Vasseur *et al.*, 1950; Tyler, 1950, 1953).[1]

The observations made on eggs subjected to basic dyes or proteins were carried out in order to get further information about the changes responsible for the described difference in interaction between spermatozoa and jelly coat. The result was that in the resting oocytes and such undergoing meiotic divisions, some masking substance protects the jelly coat both against the basic substance and against the depolymerizing action of the spermatozoa. The jelly contains numerous acid groups, as shown by Runnström *et al.* (1942). Moreover, Vasseur (1948) found that the acid group has the character of sulfate, esterified with a polysaccharide moiety, constituting about 80% of the jelly (see further Tyler, 1949). It seems thus probable that a basic substance, most likely a protein, with basic groups, would be present in the jelly coats of the oocytes including the stage of meiosis. This substance would be able to mask the sulfated polysaccharides against the action of the spermatozoa. It would be expected that the sulfated polysaccharides combined with basic proteins would be masked against the action of externally added basic substances.

Another possibility emerges from the data concerning the action of certain added inhibitors, as, for example, the *Fucus* inhibitor, chondroitin sulfate, heparin, germanine etc. (Wicklund, 1954; Runnström, 1957a). The addition of these substances brings the mature eggs into a state similar to that of underripeness. The spermatozoa are accumulated in the jelly coat, the rate of fertilization is very delayed, the fertilization membrane is not, or only asymmetrically, elevated; the ensuing cleavage is irregular. This is not only due to an effect of the jelly layer; even in eggs without jelly coats irregularities in membrane elevation may remain (Runnström and Hagström, 1955); the rate of fertilization increases but does not always attain the value prevailing in the non-treated mature eggs (Runnström, 1957a).

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[1] The papers by Collier (1959) and by Haino and Dan (1961) show that even in dilute solution the jelly coat substance induces an acrosomal reaction that is much in excess of the one found in normal sea water. Haino and Dan made the interesting observation that the action of the jelly coat substance is dependent on the age of the spermatozoa. Furthermore, early in the breeding season the reaction was weaker than at its optimum part. This is rather similar to what has been said above about the seasonal dependence of the jelly-dissolving action of the spermatozoa, although above the interaction was considered more from the point of view of the egg than from that of the spermatozoon. The data suggest, however, the working hypothesis that acrosomal reaction and activation of the jelly-depolymerizing enzyme are closely connected events. In an earlier paper Dan (1954) showed that agglutination and acrosomal reaction in spermatozoa should be considered as two separate responses of the spermatozoa to the jelly substance. Runnström and Hagström (1955) showed that the "*Fucus* inhibitor" (for references, see Metz, 1961) inhibits both the jelly-depolymerizing action of the spermatozoa and their complete separation after agglutination. This suggests that the separation is due to the action of the jelly-depolymerizing enzyme of the spermatozoa. When the spermatozoon has attached itself definitely to the cytoplasmic surface of the egg, the cortical changes start even if the further penetration of the spermatozoon is prevented (Runnström, 1957b).

The reaction of the spermatozoon with the jelly coat and that with the cytoplasmic surface of the egg are probably mediated by different sites in the acron region of the spermatozoon as may be inferred from Perlmann's work (1956, 1957, 1959) and from work of Baxandall, Perlmann and Afzelius now in press. The reactions in the surface may be of several kinds as follows also from a paper by Runnström and Kriszat (1960) that indicates the presence of a reception and an activating system in the egg surface.

The added inhibitors probably cross-link the micelles of the jelly coat. They may further inhibit the jelly-dissolving sperm-enzyme in competition with the jelly substance and in this way block or delay the penetration of the spermatozoa through the jelly coat. It is of interest that jelly substance added in relatively high concentration to the egg suspension also inhibits the sperm penetration (Runnström and Wicklund, 1950). As follows particularly from the work of Hagström (1956a, 1956b, 1956c) the jelly coat substance is usually a barrier for the spermatozoa. This barrier can only be broken down by certain of the spermatozoa. The jelly coat exerts thus a sieve-like, selecting action on the spermatozoa but, as follows also from Perlmann's observations referred to above, the selecting effect decreases with progress of the breeding season. Perlmann (1956, 1957, 1959) prepared antibodies against the jelly coat of *Paracentrotus*. The anti-jelly antibodies precipitated the jelly coat in a way very similar to the effect of basic protein. The antigens of the jelly coat are distinct from the egg antigens, although substances from the egg may enter the jelly coat, probably through a sort of secretion of diffusion. Perlmann's (1956) observations showed that the reaction of the jelly coat against anti-jelly serum varied but seemed to be extremely well correlated with the ripeness of the eggs. The oocytes only rarely showed any precipitation reaction. Perlmann explained the increased reactivity of the jelly coat to its specific antibodies by an unmasking of antigenic groups during maturation. Moreover, the reaction between jelly layer and anti-jelly serum is very low when the batch of eggs used in the experiment contained a relatively high percentage of oocytes. Even if the eggs have undergone their maturation divisions a batch of this character belongs to the underripe type. Perlmann infers from his results that the reaction between jelly and anti-jelly serum is causally related to an increased capacity of the jelly coat for letting homologous sperm pass. The maximum immune response was obtained during the optimum period in June referred to above.

It is obvious that there is a parallelism between reactivity of the jelly coat to anti-jelly serum and its accessibility to the jelly-dissolving sperm enzyme. Both sites of activity seem to be masked by the same substance or complex of substances. The parallelism can be extended to the basic substances, dyes and protamin sulfate. They do not precipitate the jelly coats of oocytes and maturing eggs but those of mature eggs. According to personal communication, Perlmann tested in 1956–1957 the effect of different sulfated polysaccharides, chondroitin sulfate, dextran sulfate, etc. on the reaction between the jelly and anti-jelly serum. No inhibition of the immune reaction was demonstrated. Conversely, the added sulfated compounds have a delaying effect on fertilization depending to a large extent on the increased resistance of the jelly coat. The spermatozoa do not constitute the limiting factor in experiments with sulfated polysaccharides. The motility and life span of the spermatozoa are considerably prolonged in the presence of the mentioned substances.

As already suggested, the addition of the sulfated compounds may increase the cross-linking within the jelly coat. This may increase the resistance to the sperm penetration that prevails even under normal conditions. Moreover, after the addition of the foreign substances the jelly-dissolving enzyme of the spermatozoa may meet a substrate beyond its range of specificity. Basic substances have not yet been studied with respect to eggs made refractory by added sulfated compounds.

More work is needed before definite conclusions can be drawn, but it is evident that, in oocytes, the reaction both between jelly and basic substances, and between jelly and anti-jelly serum are blocked. The simplest explanation is that the reacting sites are masked by basic proteins, secreted from the eggs. Maturation should then imply that the basic proteins in the jelly coat are gradually broken down. In fact, the maturation of the egg finally leads to a dissolution of the vitelline membrane which is an extremely sensitive indicator of proteolytic activity (see Runnström and Kriszat, 1960).

In fact, Lundblad (1950, 1954) found that proteolytic enzymes may be present in the jelly substance and they may even be released to the medium. Lundblad (1950) first believed that the proteolytic enzymes were components of the jelly coat but later arrived at the conclusion that the proteolytic enzymes leak out from the surface of the unfertilized egg. The enzyme mainly involved is E 2, an enzyme with its pH optimum about neutral reaction. This enzyme is inhibited by SH-compounds (Lundblad, 1954). It is activated upon fertilization but may possibly be deactivated by the release of a compound with free SH-groups. As long as E 2 dominates, the fertilization membrane is still connected with underlying structures. Moreover, the jelly coat remains dense and captures spermatozoa. The release of an SH-compound would, however, inhibit E 2, but bring about an activation of E 1 and E 3 that are present in the egg surface. In *Pa. lividus*, the changed situation begins about 30 seconds after insemination. It brings about the smoothing of the fertilization membrane, starting on the proximal side (Runnström, 1962). Moreover, a swelling of the jelly coat occurs so as to release the captured spermatozoa. This change follows closely the smoothing of the fertilization membrane (see Fig. 5). Oxidation of the SH-groups in the egg surface with the high potential dye, porphyrindin, prevents the swelling of the jelly coat and the elevation of the fertilization membrane. The spermatozoa remain captured (Runnström, 1957b). Conversely, treatment of eggs with glutathione increases the elevation of the fertilization membrane and the swelling of the jelly. It seems thus probable that several substances are exuded from the egg upon fertilization and even invade the jelly coat.

Immers (1961c) showed in eggs of *Echinus esculentus* that sulfated mucopolysaccharides escape from the egg surface concurrently with the formation of a wider perivitelline space between fertilization membrane and egg surface. Ishihara (personal communication) has elaborated these observations for other species; moreover, he demonstrated that the acid polysaccharides are chemically of a type different from those present in the jelly coat (see also Immers, 1952). Probably there may, besides the acid polysaccharides, also be other substances exuded, of which some may be held back by the fertilization membrane. Others may penetrate this latter and are thus able to act on the jelly coat.

The acid formation accompanying the release of the acid polysaccharides (Runnström and Immers, 1956; Aketa, 1963; Ishihara, personal communication) may also serve as an activator of enzymes like cathepsin II (Lundblad, 1954) that have a rather low pH-optimum.

Borei (1948) found, in work with *Ps. miliaris* from the vicinity of Kristineberg, that a relatively high rate of respiration prevailed in the unfertilized eggs immediately after their removal from the ovary. When the eggs remained in contact with sea water, the rate of respiration gradually decreased until the attainment of an asymptotic value. The post-fertilization rate of respiration was always the same irrespective of the rate prevailing before fertilization. Yasumasu and Nakano (1963), Ohnishi and Sugiyama (1963) found, however, a constant low rate of respiration in the unfertilized sea urchin egg, even when the interval between removal of the eggs from the ovaries and the fertilization was brief.

As Ohnishi and Sugiyama point out, Borei's results have thus not general validity. They are certainly to be explained by the mild type of underripeness mentioned in section 3 a. B. Hagström (1955) found that the eggs usually attain their maximum rate of fertilization 3–4 hours after removal from the ovary. In good agreement with this Borei found that the respiratory curve became almost horizontal four hours after removal of the eggs from the ovary. According to T. Hultin (personal communication) the incorporation of labeled amino acids was usually higher in unfertilized eggs of *Ps. miliaris* (Kristineberg) than in unfertilized eggs from the high season of *Pa. lividus* (Naples). In underripe eggs, low rate of fertilization seems to be combined with rather high rate of respiration and of incorporation of labeled isotopes. In certain species and under optimal external conditions the transition state may be very short.

The controversy may partly depend on variations in the rate of decline of respiration from the high level found in oocytes (Lindahl and Holter, 1941) and the low level prevailing in mature unfertilized eggs. In different species and under different conditions, the decline may be steep or more gradual.

Possibly induction of spawning by means of KCl-injection is more selective with respect to ripe eggs than the removal of the ovaries into sea water, the method used by the Swedish workers. Hultin and Hagström (1956) found that the eggs of one female of *Ps. miliaris* (Kristineberg) sometimes formed several statistical groups probably representing different stages of ripeness.

The spermatozoa enter without difficulty into the jelly coat of the oocyte that has been exposed to protamin sulfate. This suggests that the jelly coat may be perforated by radially directed pores. As recalled above thin filopodia penetrate the jelly coat in the oocyte of first order. The filopodia are withdrawn prior to the meiotic divisions. On the other hand, filopodia and reception cones appearing subsequent to insemination were sometimes seen to coexist. There may be a certain reorganization of the fine structure of the jelly coat following the meiotic divisions. The gradual maturation of the jelly coat and cytoplasm represents steps of this reorganization. After the meiotic divisions the precipitation of the jelly coat with basic substances delays considerably, or may even completely bar the penetration of spermatozoa. At the same time there is an increased tendency to formation of fibers that have a tangential direction. The changes referred to might indicate a rearrangement of micelles within the jelly coat from a more radial to a more tangential orientation.

Under anaerobic conditions the dyes are reduced within the jelly coat. The capacity of reduction of the jelly coat seems to be greater in oocytes than in mature eggs. The present observations give, however, no clue to the nature of the reducing systems. It is of interest that the degree of swelling of the jelly coat is dependent on the oxidation-reduction state of the dye, a situation that may be found also within the cytoplasm (diphosphopyridine nucleotide being a base in oxidized but not in reduced state).

Some incidental results obtained may also be briefly commented on. Treatment of unfertilized eggs with basic proteins was more injurious than a similar treatment initiated only some minutes after fertilization. The explanation is probably that, before fertilization, acid polysaccharides are bound to the egg surface and that they are, to a large extent, released from the egg surface upon fertilization (see Immers, 1961c). It is conceivable that the basic substances interfere more seriously in the former case. After fertilization the main part of the acid polysaccharides is found in extraneous structures, the fertilization membrane, the hyaline layer and the perivitelline space. Even if these structures react to fuller extent with the basic proteins, their effect on the development is small. This may be due to the continuous secretion process occurring in the surface of the embryos (Immers, 1961c). The tendency to animalization apparent in eggs treated with basic protein before fertilization indicates that the vegetal region has a differential susceptibility to the effect of the precipitation of acid polysaccharides. This may be in keeping with the view expressed by Motomura (1960) and Immers (1961a, 1961b) regarding the importance of mucopolysaccharides in the primary invagination of the archenteron.

The results of the present research show how dependent the properties of extraneous coats may be of the phase of development of the female gametes. The properties of the coats, on the other hand, may be of great importance for the relations of a cell to the surrounding medium, including other cells. The brief remarks in the preceding paragraph point to the importance of the properties of the cell coats in morphogenesis (for further references, see Gustafson and Wolpert, 1963).

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SUMMARY

1. The spermatozoa are able to depolymerize the jelly coat of mature eggs of sea urchins (*Psammechinus microtuberculatus, Ps. miliaris, Paracentrotus lividus, Arbacia lixula*), whereas that of resting oocytes or oocytes in meiosis is resistant. As follows from a survey of previous work (see Introduction) the depolymerizing

action of the spermatozoa is probably of enzymatic nature. The mechanical resistance of the jelly coat was studied by filtration of the female gametes through gauze of appropriate mesh width. The jelly coats of the oocytes proved to be more resistant than those of mature eggs.

2. The state of the jelly coat does not change abruptly at the conclusion of the meiotic divisions but a transition state prevails for a varying length of time. This is the state of cytoplasmic underripeness, the duration of which depends on exterior conditions and probably also on the species. The ripe state involves a maximum rate of fertilization and—as may be inferred from Borei's work (1948) —a minimum rate of respiration.

3. Basic dyes (toluidine blue and brilliant cresyl blue) and protamin sulfate cause a strong precipitation and contraction of the jelly coat of fertilized egg. The effect is less pronounced in unfertilized mature eggs and very weak in resting oocytes and oocytes in meiosis. It is inferred that in the oocytes there is a masking of the acid groups of the jelly coat. The masking declines only gradually according to the rate of cytoplasmic ripening that is connected with a release of certain substances from the egg cytoplasm. These influence the jelly coat. Particularly a change of the jelly coat occurs concurrently with the smoothing of the fertilization membrane. There is a parallelism between the masked state in the oocytes described above and their lack of reactivity with anti-jelly serum that was found by Perlmann (1956, 1957, 1959). He stated that the precipitating effect of the anti-jelly serum had a maximum when the eggs were in their highest state of ripeness (maximum rate of fertilization).

4. If basic dyes precipitating the jelly coats are reduced, a swelling of the jelly coats occurs. Eggs subjected to basic proteins before fertilization have a less normal development than eggs treated by the same dose of basic proteins after fertilization.

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